

Antibiotic Assay Medium No. 5 (Streptomycin Assay Agar w/ Yeast Extract), Granulated

GM006

Antibiotic Assay Medium No.5 (Streptomycin Assay Agar w/ Yeast extract), granulated is used for microbiological assay of Dihydrostreptomycin, Framycetin and Kanamycin B using *Bacillus subtilis*

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue (Peptone)	6.000
Beef extract	1.500
Yeast extract	3.000
Agar	15.000
Final pH (at 25°C)	7.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.50 grams in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates or dispense as desired.

Advice: Recommended for the Microbiological assay of Dihydrostreptomycin, Framycetin and Kanamycin B.

Principle And Interpretation

This medium is commonly used for assaying Streptomycin by cylinder plate method using *Bacillus subtilis* as test organism. This method is used in the assay of commercial preparations of antibiotics, as well as for antibiotics in body fluids, feeds etc. Medium composition is in accordance to the specifications detailed in the FDA (1) and numerically identical to the name assigned by Grove and Randall (2).

Peptic digest of animal tissue, yeast and beef extract provides necessary growth nutrients for the test organisms like *Bacillus subtilis*.

The medium provides solidified substratum for growth of organisms. The pH-7.9 maintained in this medium- provides optimum growth conditions for *Bacillus subtilis* (3). This medium is used to prepare the base as well as seed layer in the microbiological assay of antibiotics such as Dihydrostreptomycin, Framycetin and Kanamycin B.

To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized test culture can be overlaid. Even distribution of the layer is important.

Quality Control

Appearance

Cream to yellow coloured granular medium

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 2.55% w/v aqueous solution at 25°C. pH : 7.9±0.2

pH

7.70-8.10

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Cultural Response <i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant	>=70%	Dihydrostreptomycin, Framycetin, Kanamycin B

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Reference

1. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436, Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242-259, (April).
2. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
3. Stearn and Stearn, J Bacteriol. 1933. 26(1): 37-55.

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